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(54) PROCESS FOR PRODUCING TRANSFORMED CELL

(57) A process for producing transformed cells by introducing foreign genes into target cells through piercing, which comprises the step of culturing the target cells having the foreign genes injected thereinto in the presence of a cell adhesion-active substance; and a kit for producing transformed cells suitable for use in the above method and containing as the essential ingredients the cells to be transformed with foreing genes by this method and a cell adhesion-active substance.

Description

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TECHNICAL FIELD

The present invention relates to a method for production of transfected cells, more particularly, a method which makes possible to effectively transfer a foreign gene into target cells in the field such as cell technology, genetic engineering, developmental engineering and the like.

BACKGROUND ART

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As a method for transferring a foreign gene into target cells, there are known a calcium phosphate method, a DEAE-dextran method, a liposome method, an electroporation method, a microinjection method, a particle gun method and the like. All of these methods have advantages and disadvantages in respect of manipulation procedures, efficacy, damage on cells and the like. Among these methods, a perforation method such as an electroporation method, a microinjection method, a particle gun method and the like can easily handle cells without using special reagents and have good transfer efficacy. However, damage of cells by perforation can not be avoided.

The object of the present invention is to provide a method for improving the transfer efficacy when a foreign gene is transferred into target cells by a perforation method to produce transfected cells.

SUMMARY OF THE INVENTION

The first aspect of the present invention relates to a method for production of transfected cells and is characterized in that said aspect includes a step of, after injection of a foreign gene into target cells using a perforation method, culturing the cells in the presence of a cell-adhering active substance, in a method for production of a transfected cell using a perforation method.

The second aspect of the present invention relates to gene-transferred cells which are produced by the method of the present invention.

The third aspect of the present invention relates to a kit for production of transfected cells, which is used for a method for production of transfected cells according to the first aspect of the present invention and is characterized in that said aspect contains a cell-adhering active substance.

DETAILED DESCRIPTION OF THE INVENTION

The method of the present invention is characterized in that, after a foreign gene is transferred into target cells using a perforation method, the cell is cultured in the presence of a substance having the cell adhesive activity.

As used herein, the perforation method means a method for injection of a gene by perforating a cell wall, including an electroporation method, a microinjection method, a particle gun method and the like. The electroporation method is as described in, for example, Tanpakushitsu, Kakusan, Koso, volume 31, page 1591-1603 (1986). The microinjection method is as described in, for example, Cell, volume 22, page 479-488 (1980). The particle gun method is as described in, for example, Technique, volume 3, page 3-16 (1991). These methods include the known methods used for transferring a gene into cells.

For cells used in these perforation methods, for example, animal cells may be prepared according to a known method ["Shin-Seikagaku Jikkenkoza 18, Saibobaiyogijyutsu", 1st edition (1990), edited by Nippon Seikagakugakkai, published by Tokyo Kagakudojin] or cultured animal cells may be used.

As used herein, a cell-adhering active substance refers to a substance having the cell-adhering activity, that is, the activity to make target cells adhere to a cell, or to an extracellular matrix which is a substance filling a space between cells in the tissue, or to a material such as plastic, glass and the like. In the present invention, any substances having the activity can be used as long as they give no adverse effects on transfection of target cells. Such the activity is to fix cells, for example, to a culture wear covered with a cell-adhering active substance while maintaining the cell in its form, or in the spreaded form, that is, in the changed form after the cell has been spreaded in one or more directions.

Attachment between the cell-adhering active substance and the target cell can be assayed using a conventional method. The method includes, for example, a method described in Nature, 352: 438-441 (1991). Briefly, the cell-adhering active substance covers a plastic dish and a population of cells to be assayed is put into medium, allowing to stand for 30 minutes to 2 hours. After this incubation period, non-adhered cells are recovered, counted and assayed for viability. Cells adhered to the cell-adhering active substance are recovered using trypsin or a cell dissociation buffer (for example, Gibco), counted and tested for viability. Then, a proportion of adhered cells is calculated and compared with standard or standard control such as a plastic dish covered with bovine serum albumin (BSA). A combination of cell-adhering active substance/cell can be determined by substantial adhesion of the target cell with the cell-adhering active substance assayed. In addition, the cell-spreading activity can be determined by observing under a microscope a

change in the form before adhered cells are dissociated using trypsin or a cell dissociation buffer, in the above procedures.

Examples of the cell-adhering active substance include, for example, a cell-adhering active polypeptide or a functional equivalent thereof and a cell-adhesive synthetic polymer.

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Examples of the polypeptide, used in the present invention, having the cell-adhering activity include a cell-adhering active polypeptide such as invasin, polylysine and the like other than that derived from extracellular matrix, for example, a polypeptide showing the cell-spreading activity described in JP-A 2-311498, for example, components of an extracellular matrix such as fibronectin, laminin, collagen, vitronectin, osteopontin, thrombospondin, tenasin and the like. The extracellular matrix components can be prepared from a natural or cultured source by the known method [International Journal of Cancer, volume 20, page 1-5 (1977); Journal of Biological Chemistry, volume 254, page 9933-9937, (1979); "Zoku-Seikagaku Jikkenkoza, volume 6, Saibokokaku no Kozo to Kino (Structure and Function of Cell Skeleton) (last volume). (1st edition) (1986) edited by Nippon Seikagakugakkai, published by Tokyo Kagakudojin; Cell Structure and Function, volume 13, page 281-292 (1988); Journal of Biological Chemistry, volume 264, page 18202-18208 (1989); and Journal of Biological Chemistry, volume 260, page 12240-12245 (1985)]. The cell-adhering active polypeptide may be substantially purified extracellular matrix fragments or a mixture thereof. More particularly, proteins and polypeptides having the cell-adhering activity or the cell-spreading activity, or a functional equivalent thereof may be used.

As these cell-adhering active polypeptides, substantially purified natural polypeptides, polypeptides from enzymological or chemical degradation of the natural polypeptides, or the similar polypeptides made by genetic engineering may be used. Further, materials obtained by altering these polypeptides without impairing the function, that is, the cell-adhering activity or the cell-spreading activity may be used. In the present invention, even when the amino acid sequence of a polypeptide from natural origin has deletion, substitution, addition and/or insertion of an amino acid, as long as the polypeptide has the desired cell-adhering activity or the cell-spreading activity, it is referred to as a functional equivalent of a polypeptide having the natural amino acid sequence. That is, it is known that naturally occurring proteins include proteins of which amino acid sequences have mutation such as deletion, insertion, addition, substitution and the like of an amino acid due to modification reaction in the living body after production or during purification, in addition to proteins having a change in the amino acid sequence due to polymorphism or mutation of genes encoding those naturally occurring proteins and that, regardless of these, there are proteins exhibiting the physiological and biological activity substantially equivalent to that of proteins having no mutation. Like this, even when there is a structural difference between polypeptides, as long as they share the common main functions, they are called polypeptides having the functionally equivalent activity.

This is also true where the above mutations are artificially introduced into the amino acid sequence of proteins. In this case, more variety of mutants may be made. As long as these mutants exhibit the physiological activity substantially equivalent to that of proteins having no mutation, they are interpreted to be a polypeptide having the functionally equivalent activity.

For example, in many cases, a methionine residue present at a N-terminal of a protein expressed in Escherichia coli is said to be removed by an action of methionine aminopeptidase, thus, generating both proteins having a methionine residue or those having no methionine residue depending upon the kind of proteins. However, whether or not a protein has a methionine residue dose not affect on the protein activity in many cases. In addition, it is known that a polypeptide where a certain cysteine residue is substituted with a serine residue in the amino acid sequence of human interleukin-2 (IL-2) retains the interleukin-2 activity [Science, volume 224, page 1431 (1984)].

Further, upon production of proteins by genetic engineering, it is frequently conducted that the proteins are expressed as a fused protein. For example, in order to increase an amount of an expressed protein of interest, it is conducted that the protein is expressed by adding a N-terminal peptide chain derived from other protein to a N-terminal of the protein of interest, or adding a suitable peptide chain to a N-terminal or a C-terminal of the protein of interest to facilitate purification of the protein of interest by using a carrier having the affinity to the added peptide chain.

In this respect, the related biotechnological techniques have progressed and, as the result, deletion, substitution, addition or other modification of an amino acid in a functional area of a subject can be routinely carried out. Then, the resulting amino acid sequence may be routinely screened for the desired cell-adhering activity or the cell-spreading activity according to the above method.

Polypeptides having the cell-adhering activity may be an artificial polypeptide containing, in the molecule, the amino acid sequence necessary for the cell-adhering activity, for example, the amino acid sequence may be selected from the amino acid sequence represented by SEQ ID: No. 1 (RGDS), the amino acid sequence represented by SEQ ID: No. 6 (central sequence of laminin, YIGSR). These polypeptides can be prepared in a large amount by a genetic engineering method or chemical synthesis method and may be used as a purified polypeptide.

Examples of the artificial polypeptide having, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 include a polypeptide represented by SEQ ID: NO. 7 described in JP-A 1-180900. The polypeptide can be prepared using Escherichia coli HB101/pTF1409 (FERM BP-1939) according to a method described in JP-A 1-180900. In

addition polypeptides represented by respective sequence ID numbers in the sequence list shown in Table 1 below can be prepared according to a genetic engineering method described in each specification.

In addition, a plasmid HB101/pCHV90 contained in Escherichia coli HB101/pCHV90 in Table 1 can be prepared using Escherichia coli HB101/pHD101 (FERM BP-2264) and Escherichia coli JM109/pTF7021 (FERM BP-1941) according to a method described in JP-A 5-271291.

Table 1

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Laid Open publication	SEQ ID: No.	Living bacterium (Escherichia coli)	Accession No.
JP-A 1-206998	8	JM109/pTF7021	FERM BP-1941
JP-A 1-261398	9	HB101/pTF1801	FERM P-9948
JP-A 2-97397	3	JM109/pTF7221	FERM BP-1915
JP-A 2-152990	10	JM109/pTFB800	FERM BP-2126
JP-A 2-311498	11	HB101/pCH101	FERM BP-2799
JP-A 3-59000	12	JM109/pCF406	FERM P-10837
JP-A 3-232898	13	HB101/pCE102	FERM P-11226
JP-A 4-54199	14	JM109/pTF7520 +VN-IN.TAA	FERM P-11526
	15	JM109/pTF7520 +Col ^{X1}	FERM P-11527
JP-A 5-271291	16	HB101/pCHV179	FERM P-12183
	17	HB101/pCHV90	-
	18	HB101/pCHV89	FERM P-182
JP-A 5-97698	19	JM109/pTF7520CoIV	FERM BP-5277
JP-A 5-178897	20	JM109/pYMH-CF • A	FERM BP-5278

Alternatively, artificial polypeptides having, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 can be chemically synthesized. For example, PolyRGDS described in JP-A 3-173828 can be synthesized and used. Examples of artificial polypeptides having, in the molecule, the amino acid sequence represented by SEQ ID: No.

2 include a polypeptide represented by SEQ ID: No. 4 described in JP-A 2-311498 and the polypeptide can be prepared by genetic engineering using Escherichia coli HB101/pHD102 (FERM P-10721) according to a method described in JP-A 2-311498. In addition, a polypeptide represented by SEQ ID: No. 2 may be chemically synthesized according to a method described in JP-A 3-284700.

Further, examples of artificial polypeptides having, in the molecule, the amino acid sequence represented by SEQ ID: No. 2 and the amino acid sequence represented by SEQ ID: No. 3 include a polypeptide represented by SEQ ID: No. 21 described in JP-A 2-311498 and the polypeptide can be prepared by genetic engineering using Escherichia coli HB101/pCH102 (FERM BP-2800) according to a method described in JP-A 2-311498. In addition, a polypeptide represented by SEQ ID: No. 5 described in JP-A 3-284700 is a polypeptide containing, in the molecule, the amino acid sequences of SEQ ID: No. 1 and 2 and the polypeptide can be prepared by genetic engineering using Escherichia coli HB101/pCS25 (FERM P-11339) according to a method described in JP-A 3-284700.

As described above, examples of the polypeptides used in the present invention are cell-adhering active polypeptides containing, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 and/or the amino acid sequence represented by SEQ ID: No. 2. As the polypeptide, a polypeptide obtained by covalently binding a polypeptide derived from a cell adhesion domain of human fibronectin ["Fibronectin", page 47-121 (1989), edited by Mosher, D.F., published by Academic Press) with a CS1 polypeptide derived from the same (ibid), a polypeptide derived from a heparin binding domain (ibid) containing a CS1 polypeptide, or a polypeptide derived from cell adhesion can be used, and they can be made by genetic engineering, respectively. For example, respective necessary regions are taken out from a vector containing a DNA encoding a cell adhesion domain-derived polypeptide, a vector containing a DNA encoding a CS1 polypeptide, and a vector containing a DNA encoding a heparin binding domain-derived peptide containing a CS1 polypeptide, respectively, and they can be used alone or in combination thereof to make a vector expressing a polypeptide containing, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 and/or the amino acid sequence represented by SEQ ID: No. 2.

When a polypeptide where a polypeptide containing, in the molecule, the amino acid sequence represented by SEQ ID: No. 1 and a polypeptide containing, in the molecule, the amino acid sequence represented by SEQ ID: No. 2 are covalently bound is made, a covalent bonding between polypeptides may be a direct bonding or an indirect bonding, for example, an indirect bonding via a spacer. A spacer is an insertion sequence for adjusting an intermolecular distance in each region. As the spacer, an arbitral peptide chain can be used, for example, a sequence upstream of a CS1 region in fibronectin molecule. The spacer sequence can be easily introduced therein by genetic engineering.

The cell-adhesive synthetic polymers include the known poly-N-p-vinylbenzyl-D-lactoneamide (PVLA).

In the present invention, the target cell include, but being not limited to, hematopoiesis stem cell, peripheral blood stem cell, umbilical blood cell, ES cell, lymphocyte, cancer cell and the like.

Examples of the foreign gene include, but being not limited to, nucleic acid selected from nucleic acids encoding proteins, nucleic acids encoding polypeptides, antisense DNA's, antisense RNA's, ribozymes, nucleic acids encoding intracellular antibodies and pseudogenes (decoy genes). In the present invention, the foreign gene may be inserted into a vector.

Examples of the vector are retrovirus vector, adenovirus vector, vacciniavirus vector, herpesvirus vector and the like.

According to the present invention, a target cell into which a foreign gene has been transferred by a perforation method according to a conventional method can be cultured in the presence of a cell-adhering active substance to effectively obtain transfected cells with a transferred gene. A cell culture method may be selected from the known methods depending upon a cell used. For example, when cell culturing is performed in the presence of a cell-adhering active polypeptide, 250 to 2000 μ g/ml of the cell-adhering active polypeptide may be used in a culture medium to culture it according to a conventional method.

Particularly, culturing is preferably carried out using a culture wear covered with a cell-adhering active substance. The culture wear refers to any wear normally used for cell culture, for example, a culture dish, a culture wear using a microcarrier, and a culture wear using fibrous hollow fibers. The culture wear may be covered with the substance by coating or spraying. For example, the culture wear may be easily covered with the cell-adhering active substance. The culture wear may be easily covered with the polypeptide by dissolving it in a suitable solution such as a phosphate buffered saline (PBS), adding the solution to the culture wear and allowing to stand for a suitable period of time. An amount of the polypeptide with which the culture wear is covered may be selected from a range of 50 to 1000 pmol/cm², suitably 150 to 600 pmol/cm².

Transfected cells which have been cultured in the presence of the cell-adhering active substance can be obtained from a culture according to a conventional method. Thus, transfected cells can be produced effectively.

The resulting transfected cells are useful for production of useful substances by cells using gene recombination techniques, exploitation of disease models, gene therapy and the like. Thus, transfected cells can be effectively produced according to the present invention.

In addition, the present invention can be simply carried out by using a kit containing a cell-adhering active substance. The cell-adhering active substance to be contained in the kit may be in a form of solutions or lyophilized powders. The kit may contain a buffer for dissolving or diluting the cell-adhering active substance, a cell culture medium, a cell culture wear and the like. For example, a transfected cell can be simply produced by preparing a kit combining polypeptides, PBS for diluting the polypeptide, a cell culture wear and the like which are used for the method of the present invention. A reagent contained in the kit may be liquid or lyophilized.

A perforation method in the present invention can be used by appropriately selecting from an electroporation method, a microinjection method, a particle gun method and the like depending upon the purpose.

The present invention is illustrated by Examples below but is not limited to them.

45 Example 1

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1. Coating of cell-adhering active polypeptide on culture dish

A polypeptide represented by SEQ ID: No. 3 (hereinafter referred to as "C274"), a polypeptide represented by SEQ ID: No. 4 (hereinafter referred to as "H296") and a polypeptide represented by SEQ ID: No. 5 (hereinafter referred to as "C \cdot CS1") were dissolved in a phosphate buffered saline (PBS) to each 1 μ M, respectively, which were steriled using a 0.22 μ m filter (Millex-GV, Millipore).

Each 1 ml/well of these solutions was added to a 24-well polystyrene culture dish (manufactured by Corning), respectively, to coat the dish at 4 °C overnight. These dishes were rinsed with a 500 μ l/well of a Dulbecco's modified minimum basal medium containing no bovine fetal serum prior to addition of a transformed cell described below.

2. Transfection of cells

Two culture dishes (diameter: 100 mm) of human epidermoid cancer cell A-431 which had been cultured in a Dul-

becco's modified minimum basal medium containing 10% bovine fetal serum were rinsed with 10 ml of a Dulbecco's modified minimum basal medium containing no bovine fatal serum, respectively, and 3 ml of PBS containing 0.25% bovine trypsin and 0.02% EDTA was added thereto to detach cells from the culture dish. To these was added 7 ml of a Dulbecco's modified minimum basal medium containing no bovine fetal serum, followed by centrifugation at 800 rpm for 3 minutes to collect cells. The resulting cells were suspended in 10 ml of a Dulbecco's modified minimum basal medium containing bovine fetal serum, followed by centrifugation at 800 rpm for 3 minutes to collect cells. The resulting cells were combined, suspended in 10 ml of PBS, a 3/10 aliquot of the suspension was taken and divided into two equal aliquots, which were centrifuged at 800 rpm for 3 minutes to collect cells, respectively. The resulting cells were suspended again in 10 ml of PBS, followed by centrifugation at 800 rpm for 3 minutes to collect two batches of cells. One batch of the resulting cells were suspended in 1 ml of PBS containing 15 µg of pCAT-control vector (Promega) which had been aseptically prepared, and placed in an electroporation cuvette for Gene Pulser (BioRad), which were allowed to stand in ice for 10 minutes. The other batch of the resulting cells were suspended in 1 ml of PBS, and placed in an electroporation cuvette for Gene Pulser (BioRad), which were allowed to stand in ice for 10 minutes. Each batch of cells were allowed to stand in ice for 10 minutes, and voltage was applied thereto at 250V and 960 µF. After application, the cells were allowed to stand in a cuvette in ice for 10 minutes. Thereafter, the cells were recovered into 15 ml of a Dulbecco's modified minimum basal medium containing 10% bovine fetal serum, 1 ml/well of which were added to a 24-well polystyrene culture dish covered with the above polypeptide. These cells were cultured at 37 °C in the presence of 5% CO₂ gas overnight, the medium was removed by aspiration, and 1 ml/well of a fresh Dulbecco's modified minimum basal medium containing 10% bovine fetal serum was added thereto, followed by culturing at 37 °C in the presence of 5% CO₂ gas overnight.

3. Determination of transfection efficacy (efficacy of gene transfer)

The cultured cells were rinsed three times with 1.25 ml of PBS per well, a lysed cell solution was prepared, and detection of expressed CAT was carried out using CAT-ELISA kit (manufactured by Boehringer Mannheim) according to a method for using the present kit. Since the present kit used a horseradish peroxidase-labelled secondary antibody and ABTS as a substrate, a ratio of 405nm/490nm was determined. An value obtained by subtracting a blank value from a value for each group in a case of addition of pCAT-control vector using as a blank a group in a case of no addition of pCAT-control vector upon electroporation was adopted as an amount of expressed CAT.

The results thereof are shown in Fig. 1. That is, Fig. 1 is a view showing efficacy of gene transfer into a cell in each polypeptide-treatment group, where the ordinate shows non-treated group and each polypeptide-treatment group and the abscissa shows gene transfer efficacy expressed as a ratio of absorbance at 405 nm relative to that at 490 nm.

As shown in Fig. 1, an amount of expressed CAT in the culture dish in the C274, H296 or C • CS1-treatment group is higher as compared with that in a non-treatment group, demonstrating that efficacy of transfer of pCAT-control vector into a cell is higher.

Example 2

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1. Coating of cell-adhering active polypeptide on culture dish

A polypeptide represented by SEQ ID: No. 3 (hereinafter referred to as "C274"), a polypeptide represented by SEQ ID: No. 4 (hereinafter referred to as "H296") and a polypeptide represented by SEQ ID: No. 5 (hereinafter referred to as "C \cdot CS1") were dissolved in a phosphate buffered saline (PBS) to each 1 μ M, respectively, which were steriled using a 0.22 μ m filter (Millex-GV, Millipore). 1 ml/well of these solutions were added to a 24-well polystyrene culture dish (manufactured by Corning) to coat the dish at 4 °C overnight, respectively. These dishes were rinsed with 500 μ l/well of a Dulbecco's modified minimum basal medium containing no bovine fetal serum prior to addition of a transformed cell described below.

2. Transfection of cell

Two culture dishes (diameter: 100 mm) of African green monkey kidney cell COS-7 which had been cultured in a Dulbecco's modified minimum basal medium containing 10% bovine fetal serum were rinsed with 10 ml of a Dulbecco's modified minimum basal medium containing no bovine fatal serum, respectively, and 3 ml of PBS containing 0.25% bovine trypsin and 0.02% EDTA was added thereto to detach cells from the culture dish. To these was added 7 ml of a Dulbecco's modified minimum basal medium containing no bovine fetal serum, respectively, followed by centrifugation at 800 rpm for 3 minutes to collect cells. The resulting cells were suspended in 10 ml of a Dulbecco's modified minimum basal medium containing bovine fetal serum, followed by centrifugation at 800 rpm for 3 minutes to collect cells. The resulting cells were combined, suspended in 12 ml of PBS, a 5/6 aliquot of the suspension was taken and divided into two equal aliquots, which were centrifuged at 800 rpm for 3 minutes to collect cells, respectively. The resulting cells

were suspended in 6 ml of PBS, followed by centrifugation at 800 rpm for 3 minutes to collect two batches of cells. One batch of the resulting cells were suspended in 1 ml of PBS containing 15 μ g of pCAT-control vector (Promega) which had been aseptically prepared, and placed in an electroporation cuvette for Gene Pulser (BioRad), which was allowed to stand in ice for 10 minutes. The other batch of the resulting cells were suspended in 1 ml of PBS, and placed in an electroporation cuvette for Gene Pulser (BioRad), which was allowed to stand in ice for 10 minutes. Each batch of cells were allowed to stand in ice for 10 minutes, and voltage was applied thereto at 250V and 960 μ F. After application, the cells were allowed to stand in a cuvette in ice for 10 minutes. Thereafter, the cells were recovered into 15 ml of a Dulbecco's modified minimum basal medium containing 10% bovine fetal serum, 1 ml/well of the cells were added to a 24-well polystyrene culture dish covered with the above polypeptide. These cells were cultured at 37 °C in the presence of 5% CO₂ gas overnight, the medium was removed by aspiration, and 1 ml/well of a fresh Dulbecco's modified minimum basal medium containing 10% bovine fetal serum was added, followed by culturing at 37 °C in the presence of 5% CO₂ gas overnight.

3. Determination of transfection efficacy (efficacy of gene transfer)

The cultured cells were rinsed three times with 1.25 ml of PBS per well, a lysed cell solution was prepared, and detection of expressed CAT was carried out using CAT-ELISA kit (manufactured by Boehringer Mannheim) according to a method for using the present kit. Since the present kit used a horseradish peroxidase-labelled secondary antibody and ABTS as a substrate, a ratio of 405nm/490nm was determined. An value obtained by subtracting a blank value from a value for each group in a case of addition of pCAT-control vector using as a blank a group in a case of no addition of pCAT-control vector upon electroporation was adopted as an amount of expressed CAT. The results thereof are shown in Fig. 2. That is, Fig. 2 is a view showing efficacy of gene transfer into a cell in each polypeptide-treatment group, where the ordinate shows non-treated group and each polypeptide-treatment group and the abscissa shows gene transfer efficacy expressed as a ratio of absorbance at 405 nm relative to that at 490 nm.

As shown in Fig. 2, an amount of expressed CAT in the culture dish in the above C274, H296 or C • CS1-treatment group is higher as compared with that in a non-treatment group, demonstrating that efficacy of transfer of pCAT-control vector into a cell is higher.

Example 3

Preparation of kit

A kit for production of gene-transfered cells was made from C274, H296, C \cdot CS1, PBS and a culturing dish as shown in Table 2 below. Reagents A, B and C were prepared so that the above polypeptides were adjusted with PBS to indicated concentrations shown in the Table. Other components were used which are described in Example 1. In addition, all of reagents A, B and C and a diluent for reagents were aseptically prepared by pre-filtering with a 0.22 μ m sterile filter.

Table 2

Kit for production of transfer	ted cell
Reagent A • • • 100 μM C274	150 µl
Reagent B • • • 100 µM H296	150 μΙ
Reagent C • • • 100 µM C • CS1	150 μΙ
Diluent for reagents · · · PBS	45 ml
24-well polystyrene culture dish	3

As described above, the present invention can overcome the problems of the previous methods for gene transfer into cells and provide a method, for production of transfected cells, having improved efficacy of gene transfer into target cells. The present invention can also provide a kit, for production of transfected cells, which are used for the method.

BRIEF DESCRIPTION OF DRAWINGS

Fig. 1 is a graph showing the effect of cell-adhering active polypeptide treatment on gene transfer efficacy in transfer of pCAT-control vector into human epidermoid cancer cell A-431.

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Fig. 2 is a graph showing the effect of cell-adhering active polypeptide treatment on gene transfer efficacy in transfer of pCAT-control vector into African green monkey kidney cell COS-7.

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Sequence Listing

(1) GENERAL INFORMATION: (i) APPLICANT: (A) NAME: Takara Shuzo Co., Ltd. (B) STREET: 609, Takenaka-cho, Fushimi-ku 10 (C) CITY: Kyoto-shi, Kyoto (E) COUNTRY: Japan (F) ZIP: 612 (ii) TITLE OF INVENTION: Method for production of transfected cells 15 (iii) NUMBER OF SEQUENCES: 21 (iv) COMPUTER READABLE FORM: (A) MEDIUM TYPE: 3.5" Diskette, 1.44 Mb 20 (B) COMPUTER: IBM PS/2 Model 50Z or 55SX (C) OPERATING SYSTEM: MS-DOS (Version 5.0) (D) SOFTWARE: Microsoft Word (v) CURRENT APPLICATION DATA: (A) APPLICATION NUMBER: EP 95 93 8599.8 25 (B) FILING DATE: (vi) PRIOR APPLICATION DATA: (A) APPLICATION NUMBER: PCT/JP95/02425 30 (B) FILING DATE: 29. November 1995 (2) INFORMATION FOR SEQ ID NO: 1: (i) SEQUENCE CHARACTERISTICS: (A) LENGTH: 4 35 (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1: 40 Arg Gly Asp Ser (2) INFORMATION FOR SEQ ID NO:2: (i) SEQUENCE CHARACTERISTICS: 45 (A) LENGTH: 25 (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide 50 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2: Asp Glu Leu Pro Gln Leu Val Thr Leu Pro His Pro Asn Leu His 5 10 Gly Pro Glu Ile Leu Asp Val Pro Ser Thr 20 55

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(2) INFORMATION FOR SEQ ID NO: 3:
              (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 274
              (B) TYPE: amino acid
5
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear
              (ii) MOLECULE TYPE: peptide
              (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:
              Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
10
                                                    10
              Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
                               20
                                                    25
              Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                               35
                                                    40
15
              Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
                               50
                                                    55
              Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                               65
                                                    70
              His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
                               80
                                                    85
              Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                               95
                                                   100
              Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
                              110
                                                   115
              Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
25
                              125
                                                   130
              Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
                              140
                                                   145
              Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
                              1.55
                                                   160
              Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
30
                              170
                                                   175
                                                                        180
              Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
                              185
                                                   190
             Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
                              200
                                                   205
35
             Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
                              215
                                                   220
             Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
                              230
                                                   235
             Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
                              245
                                                   250
40
             Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
                              260
                                                   265
             Thr Glu Ile Asp
              (2) INFORMATION FOR SEQ ID NO: 4:
              (i) SEQUENCE CHARACTERISTICS:
45
              (A) LENGTH: 296
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear
              (ii) MOLECULE TYPE: peptide
              (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:
             Ala Ile Pro Ala Pro Thr Asp Leu Lys Phe Thr Gln Val Thr Pro
                                                   10
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Thr Ser Leu Ser Ala Gln Trp Thr Pro Pro Asn Val Gln Leu Thr
                                                    25
                               20
              Gly Tyr Arg Val Arg Val Thr Pro Lys Glu Lys Thr Gly Pro Met
                               35
                                                    40
5
              Lys Glu Ile Asn Leu Ala Pro Asp Ser Ser Ser Val Val Val Ser
                               50
                                                    55
              Gly Leu Met Val Ala Thr Lys Tyr Glu Val Ser Val Tyr Ala Leu
                                                    70
                               65
              Lys Asp Thr Leu Thr Ser Arg Pro Ala Gln Gly Val Val Thr Thr
10
                               80
                                                    85
              Leu Glu Asn Val Ser Pro Pro Arg Arg Ala Arg Val Thr Asp Ala
                               95
                                                   100
              Thr Glu Thr Thr Ile Thr Ile Ser Trp Arg Thr Lys Thr Glu Thr
                                                   115
                              110
                                                                        120
              Ile Thr Gly Phe Gln Val Asp Ala Val Pro Ala Asn Gly Gln Thr
15
                              125
                                                   130
              Pro Ile Gln Arg Thr Ile Lys Pro Asp Val Arg Ser Tyr Thr Ile
                              140
                                                   145
              Thr Gly Leu Gln Pro Gly Thr Asp Tyr Lys Ile Tyr Leu Tyr Thr
                              155
                                                   160
              Leu Asn Asp Asn Ala Arg Ser Ser Pro Val Val Ile Asp Ala Ser
20
                                                   175
                              170
                                                                        180
              Thr Ala Ile Asp Ala Pro Ser Asn Leu Arg Phe Leu Ala Thr Thr
                                                   190
                              185
                                                                        195
              Pro Asn Ser Leu Leu Val Ser Trp Gln Pro Pro Arq Ala Arg Ile
                              200
                                                   205
                                                                        210
25
              Thr Gly Tyr Ile Ile Lys Tyr Glu Lys Pro Gly Ser Pro Pro Arg
                              215
                                                   220
                                                                        225
              Glu Val Val Pro Arg Pro Arg Pro Gly Val Thr Glu Ala Thr Ile
                              230
                                                   235
              Thr Gly Leu Glu Pro Gly Thr Glu Tyr Thr Ile Tyr Val Ile Ala
                              245
                                                   250
30
              Leu Lys Asn Asn Gln Lys Ser Glu Pro Leu Ile Gly Arg Lys Lys
                              260
                                                   265
              Thr Asp Glu Leu Pro Gln Leu Val Thr Leu Pro His Pro Asn Leu
                              275
                                                   280
                                                                        285
              His Gly Pro Glu Ile Leu Asp Val Pro Ser Thr
                              290
35
              (2) INFORMATION FOR SEQ ID NO: 5:
              (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 302
              (B) TYPE: amino acid
40
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear
               (ii) MOLECULE TYPE: peptide
              (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:
              Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
45
                                                    10
              Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
                                20
                                                    25
              Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                                35
                                                    40
              Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
50
                                50
                                                    55
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Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln

	His	Glu	Ser	Thr	Pro 80	Leu	Arg	Ġly	Arg	Gln 85	Lys	Thr	Gly	Leu	Asp 90
	Ser	Pro	Thr	Gly	Ile 95	Asp	Phe	Ser	Asp		Thr	Ala	Asn	Ser	
5	Thr	Val	His	Trp	Ile 110	Ala	Pro	Arg	Ala		Ile	Thr	Gly	Tyr	
	Ile	Arg	His	His	Pro	Glu	His	Phe	Ser		Arg	Pro	Arg	Glu	
10	Arg	Val	Pro	His	Ser 140	Arg	Asn	Ser	Ile		Leu	Thr	Asn	Leu	
	Pro	Gly	Thr	Glu	Tyr 155	Val	Val	Ser	Ile		Ala	Leu	Asn	Gly	
	Glu	Glu	Ser	Pro	Leu 170	Leu	Ile	Gly	Gln	Gln 175	Ser	Thr	Val	Ser	
15	Val	Pro	Arg	Asp	Leu 185	Glu	Val	Val	Ala	Ala 190	Thr	Pro	Thr	Ser	
					Asp 200					205		_	_	-	Arg 210
	Ile	Thr	Tyr	Gly	Glu 215	Thr	Gly	Gly	Asn	Ser 220	Pro	Val	Gln	Glu	Phe 225
20	Thr	Val	Pro	Gly	Ser 230	Lys	Ser	Thr	Ala	Thr 235	Ile	Ser	Gly	Leu	Lys 240
	Pro	Gly	Val	Asp	Tyr 245	Thr	Ile	Thr	Val	Tyr 250	Ala	Val	Thr	Gly	Arg 255
					Ala 260					265				_	270
25					Lys 275					280					285
				Pro	Asn 290	Leu	His	Gly	Pro	Glu 295	Ile	Leu	Asp	Val	Pro 300
	Se	r Thi	-												
30	(i) (A)	SEQU LENG	JENCE STH:	5 CH	FOR ARACT	TERIS			5:						
35	(C) (D) (ii)	STRA TOPO MOI	PECUI PECUI	ONESS C: li LE TY	S: si inear (PE: ESCR)	ingle : pept	ide	FO T	וו אר) ·					
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40	1 1	11e	Gly	Ser	Arg 5										
	(i)	SEQU	ORMAI JENCE STH:	CHA	FOR ARACT	SEQ ERIS	ID N	10: 7 3:	<i>!</i> :						
4 5	(C)	STRA	NDEI	NESS	acio S: si	ngle	<u>:</u>								
	(ii)	MOI	ECUI	E TY	lnear PE: SCRI	pept	ide N: S	SEQ I	D NC): 7:					
50	Ala 1	Val	Pro	Pro	Pro	Thr	Asp	Leu	Arg		Thr	Asn	Ile	Gly	
	_	Thr	Met	Arg	Val 20	Thr	Trp	Ala	Pro	10 Pro 25	Pro	Ser	Ile	Asp	
															30

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35
                                                    40
              Val Ala Glu Leu Ser Ile Ser Pro Ser Asp Asn Ala Val Leu
                               50
                                                    55
              Thr Asn Leu Leu Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser
5
                               65
                                                    70
              Val Tyr Glu Gln His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys
                               80
                                                    85
              Thr Gly Leu Asp Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr
                                                   100
                                                                        105
              Ala Asn Ser Phe Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile
10
                              110
                                                   115
                                                                        120
              Thr Gly Tyr Arg Ile Arg His His Pro Glu His Phe Ser Gly Arg
                              125
                                                   130
                                                                        135
              Pro Arg Glu Asp Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu
                              140
                                                   145
15
              Thr Asn Leu Thr Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala
                                                  160
                              155
              Leu Asn Gly Arg Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser
                              170
                                                   175
              Thr Val Ser Asp Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr
                                                   190
                                                                        195
                              185
20
              Pro Thr Ser Leu Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val
                              200
                                                   205
              Arg Tyr Tyr Arg Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro
                              215
                                                   220
                                                                        225
              Val Gln Glu Phe Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile
25
                              230
                                                   235
              Ser Gly Leu Lys Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala
                              245
                                                   250
              Val Thr Gly Arg Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser
                              260
                                                   265
                                                                        270
              Ile Asn Tyr Arg Thr Glu Ile Asp Lys Pro Ser Gln Met
30
                              275
              (2) INFORMATION FOR SEQ ID NO: 8:
              (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 279
35
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear
              (ii) MOLECULE TYPE: peptide
              (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:
              Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
              Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
                               20
                                                    25
              Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                                35
                                                    40
              Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
                                50
                                                    55
              Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                                65
                                                    70
              His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
50
                                80
                                                    85
              Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                                                   100
              Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
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110
                                                   115
              Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
                               125
                                                   130
                                                                        135
              Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
5
                               140
                                                   145
                                                                        150
              Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
                               155
                                                   160
              Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
                               170
                                                   175
                                                                        180
10
              Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
                               185
                                                   190
                                                                        195
              Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
                               200
                                                   205
                                                                        210
              Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
                               215
                                                   220
15
              Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
                               230
                                                   235
              Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
                               245
                                                   250
              Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
20
                              260
                                                   265
                                                                        270
              Thr Glu Ile Asp Lys Pro Ser Gln Met
                              275
              (2) INFORMATION FOR SEQ ID NO: 9:
              (i) SEQUENCE CHARACTERISTICS:
25
              (A) LENGTH: 474
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear
              (ii) MOLECULE TYPE: peptide
              (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:
30
              Ala Val Pro Pro Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro
              Asp Thr Met Arg Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu
                               20
                                                    25
              Thr Asn Phe Leu Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp
35
                                                    40
              Val Ala Glu Leu Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu
                               50
                                                    55
              Thr Asn Leu Leu Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser
                               65
                                                    70
40
              Val Tyr Glu Gln His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys
                               80
                                                    85
              Thr Gly Leu Asp Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr
                               95
                                                   100
              Ala Asn Ser Phe Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile
                              110
                                                  115
45
                                                                       120
              Thr Gly Tyr Arg Ile Arg His His Pro Glu His Phe Ser Gly Arg
                              125
                                                  130
              Pro Arg Glu Asp Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu
                              140
                                                  145
             Thr Asn Leu Thr Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala
50
                              155
                                                  160
             Leu Asn Gly Arg Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser
                              170
                                                  175
             Thr Val Ser Asp Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr
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185
                                                  190
             Pro Thr Ser Leu Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val
                              200
                                                  205
             Arg Tyr Tyr Arg Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro
5
                              215
                                                  220
             Val Gln Glu Phe Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile
                              230
                                                  235
             Ser Gly Leu Lys Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala
                              245
                                                  250
             Val Thr Gly Arg Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser
10
                              260
                                                  265
             Ile Asn Tyr Arg Thr Glu Ile Asp Lys Pro Ser Gln Asn Glu Gly
                              275
                                                  280
             Leu Asn Gln Pro Thr Asp Asp Ser Cys Phe Asp Pro Tyr Thr Val
                              290
                                                  295
15
             Ser His Tyr Ala Val Gly Asp Glu Trp Glu Arg Met Ser Glu Ser
                              305
                                                  310
             Gly Phe Lys Leu Leu Cys Gln Cys Leu Gly Phe Gly Ser Gly His
                              320
                                                  325
             Phe Arg Cys Asp Ser Ser Arg Trp Cys His Asp Asn Gly Val Asn
                              335
                                                  340
20
             Tyr Lys Ile Gly Glu Lys Trp Asp Arg Gln Gly Glu Asn Gly Gln
                              350
                                                  355
             Met Met Ser Cys Thr Cys Leu Gly Asn Gly Lys Gly Glu Phe Lys
                              365
                                                  370
                                                                       375
             Cys Asp Pro His Glu Ala Thr Cys Tyr Asp Asp Gly Lys Thr Tyr
25
                              380
                                                  385
             His Val Gly Glu Gln Trp Gln Lys Glu Tyr Leu Gly Ala Ile Cys
                              395
                                                  400
             Ser Cys Thr Cys Phe Gly Gly Gln Arg Gly Trp Arg Cys Asp Asn
                              410
                                                  415
             Cys Arg Arg Pro Gly Gly Glu Pro Ser Pro Glu Gly Thr Thr Gly
30
                              425
                                                  430
             Gln Ser Tyr Asn Gln Tyr Ser Gln Arg Tyr His Gln Arg Thr Asn
                              440
                                                  445
             Thr Asn Val Asn Cys Pro Ile Glu Cys Phe Met Pro Leu Asp Val
                              455
                                                  460
             Gln Ala Asp Arg Glu Asp Ser Arg Glu
35
                              470
              (2) INFORMATION FOR SEQ ID NO: 10:
              (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 385
40
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear
              (ii) MOLECULE TYPE: peptide
              (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:
45
             Ala Pro Ile Val Asn Lys Val Val Thr Pro Leu Ser Pro Pro Thr
                                                    10
             Asn Leu His Leu Glu Ala Asn Pro Asp Thr Gly Val Leu Thr Val
                               20
             Ser Trp Glu Arg Ser Thr Thr Pro Asp Ile Thr Gly Tyr Arg Ile
50
                               35
                                                    40
             Thr Thr Thr Pro Thr Asn Gly Gln Gln Gly Asn Ser Leu Glu Glu
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5

Val Val His Ala Asp Gln Ser Ser Cys Thr Phe Asp Asn Leu Ser

					65					70					75
	Pro	Gly	Leu	Glu	Tyr 80	Asn	Val	Ser	Val	Tyr 85	Thr	Val	Lys	Asp	Asp 90
5	Lys	Glu	Ser	Val	Pro 95	Ile	Ser	Asp	Thr	Ile 100	Ile	Pro	Ala	Val	Pro 105
	Pro	Pro	Thr	Asp	Leu 110	Arg	Phe	Thr	Asn	Ile 115	Gly	Pro	Asp	Thr	Met 120
	Arg	Val	Thr	Trp	Ala 125	Pro	Pro	Pro	Ser	Ile 130	Asp	Leu	Thr	Asn	Phe 135
10	Leu	Val	Arg	Tyr	Ser 140	Pro	Val	Lys	Asn	Glu 145	Glu	Asp	Val	Ala	Glu 150
	Leu	Ser	Ile	Ser	Pro 155	Ser	Asp	Asn	Ala	Val 160	Val	Leu	Thr	Asn	Leu 165
	Leu	Pro	Gly	Thr	Glu 170	Tyr	Val	Val	Ser	Val 175	Ser	Ser	Val	Tyr	
15	Gln	His	Glu	Ser		Pro	Leu	Arg	Gly	Arg 190	Gln	Lys	Thr	Gly	
	Asp	Ser	Pro	Thr		Ile	Asp	Phe	Ser	Asp 205	Ile	Thr	Ala	Asn	
	Phe	Thr	Val	His		Ile	Ala	Pro	Arg	Ala 220	Thr	Ile	Thr	Gly	
20	Arg	Ile	Arg	His	His 230	Pro	Glu	His	Phe	Ser 235	Gly	Arg	Pro	Arg	
	Asp	Arg	Val	Pro	His 245	Ser	Arg	Asn	Ser	Ile 250	Thr	Leu	Thr	Asn	
25	Thr	Pro	Gly	Thr	Glu 260	Tyr	Val	Val	Ser	Ile 265	Val	Ala	Leu	Asn	Gly 270
	Arg	Glu	Glu	Ser	Pro 275	Leu	Leu	Ile	Gly	Gln 280	Gln	Ser	Thr	Val	Ser 285
	Asp	Val	Pro	Arg	Asp 290	Leu	Glu	Val	Val	Ala 295	Ala	Thr	Pro	Thr	Ser 300
30					305					Val 310			_	_	315
					320					Asn 325					330
					335					Ala 340				_	345
35					350					Val 355					360
					365					Pro 370	Ile	Ser	Ile	Asn	Tyr 375
	Arg	Thr	Glu	Ile	Asp 380	Lys	Pro	Ser	Gln	Met 385					
40	(2)	TATE	``````	77.7.1	B0.5	0.00	TD 1								
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45				mino DNES:			e								
45	(D)	TOP	DLOG	Y: 1.	inea:	r -									
	(ii (xi) MOI	LECU!	LE T	YPE: ESCR	pept IPTI	tide ON: :	SEQ :	ID N	o: 1	1:				
50			Asp	Leu	Arg	Phe	Thr	Asn	Ile	Gly	Pro	Asp	Thr	Met	
50	l Val		Trp	Ala		Pro	Pro	Ser	Ile	10 Asp	Leu	Thr	Asn	Phe	
	Val	Arg	Tyr	Ser	20 Pro	Val	Lys	Asn	Glu	25 Glu	Asp	Val	Ala	Glu	30 Leu

	Ser	Tle	Ser	Pro	35), ep	n e n	π1 ~	37 - 3	40	7	m	.	_	45
					50					55		Thr			60
5					65					70		Val	_		75
					80					85		Thr	_		90
					95					100		Ala			105
10					110					115		Thr	_	_	120
	Ile	Arg	His	His	Pro 125	Glu	His	Phe	Ser	Gly 130	Arg	Pro	Arg	Glu	Asp 135
	Arg	Val	Pro	His	Ser 140	Arg	Asn	Ser	Ile	Thr 145	Leu	Thr	Asn	Leu	
15	Pro	Gly	Thr	Glu	Tyr 155	Val	Val	Ser	·Ile		Ala	Leu	Asn	Gly	Arg 165
	Glu	Glu	Ser	Pro		Leu	Ile	Gly	Gln	Gln 175	Ser	Thr	Val	Ser	Asp 180
20	Val	Pro	Arg	Asp	Leu 185	Glu	Val	Val	Ala		Thr	Pro	Thr	Ser	Leu 195
20	Leu	Ile	Ser	Trp	Asp 200	Ala	Pro	Ala	Val		Val	Arg	Tyr	Tyr	Arg 210
	Ile	Thr	Tyr	Gly	Glu 215	Thr	Gly	Gly	Asn		Pro	Val	Gln	Glu	
25	Thr	Val	Pro	Gly	Ser 230	Lys	Ser	Thr	Ala		Ile	Ser	Gly	Leu	Lys 240
	Pro	Gly	Val	Asp	Tyr 245	Thr	Ile	Thr	Val		Ala	Val	Thr	Gly	Arg 255
	Gly	Asp	Ser	Pro	Ala 260	Ser	Ser	Lys	Pro		Ser	Ile	Asn	Tyr	Arg 270
30	Thr	Glu	Ile	Asp	Lys 275	Pro	Ser	Met	Ala	Ile 280	Pro	Ala	Pro	Thr	Asp 285
	Leu	Lys	Phe	Thr	Gln 290	Val	Thr	Pro	Thr	Ser 295	Leu	Ser	Ala	Gln	Trp 300
	Thr	Pro	Pro	Asn	Val 305	Gln	Leu	Thr	Gly	Tyr 310	Arg	Val	Arg	Val	Thr 315
35					320					325		Asn			Pro
					335					340		Val			Lys 345
	Tyr	Glu	Val	Ser	Val 350	Tyr	Ala	Leu	Lys	Asp 355	Thr	Leu	Thr	Ser	Arg 360
40	Pro	Ala	Gln	Gly	Val 365	Val	Thr	Thr	Leu	Glu 370	Asn	Val	Ser	Pro	Pro 375
,	Arg	Arg	Ala	Arg	Val 380	Thr	Asp	Ala	Thr	Glu 385	Thr	Thr	Ile	Thr	Ile 390
4 5	Ser	Trp	Arg	Thr	Lys 395	Thr	Glu	Thr	Ile		Gly	Phe	Gln	Val	Asp 405
45	Ala	Val	Pro	Ala	Asn 410	Gly	Gln	Thr	Pro	Ile 415	Gln	Arg	Thr	Ile	Lys 420
	Pro	Asp	Val	Arg	Ser 425	Tyr	Thr	Ile	Thr		Leu	Gln	Pro	Gly	Thr 435
50	Asp	Tyr	Lys	Ile	Tyr 440	Leu	Tyr	Thr	Leu	Asn 445	Asp	Asn	Ala	Arg	Ser 450
	Ser	Pro	Val	Val		Asp	Ala	Ser	Thr		Ile	Asp	Ala	Pro	Ser 465
	Asn	Leu	Arg	Phe	Leu	Ala	Thr	Thr	Pro		Ser	Leu	Leu	Val	Ser

					470					475					480
					485	Ala				490				_	495
5					500	Pro				505					510
					515	Ala				520				_	525
	Glu	Tyr	Thr	Ile	Tyr 530	Val	Ile	Ala	Leu	Lys 535	Asn	Asn	Gln	Lys	Ser 540
10	Glu	Pro	Leu	Ile	Gly 545	Arg	Lys	Lys	Thr						
15	(i) (A) (B) (C) (D) (ii	SEQUENT STRATE TOPO MODE	JENC: GTH: E: ai ANDE: DLOG' LECU:	E CH 422 mino DNES Y: 1 LE T	ARAC' acies: s: s: inea: YPE:	ingle r pept	STIC:	S:		.	2				
80	(XI) SE(วดะพ	JE D	ESCR.	IPTI)N: :	SEQ .	ID N	J: 1:	2:				
20	Pro	Thr	Asp	Leu	Arg 5	Phe	Thr	Asn	Ile		Pro	Asp	Thr	Met	
		Thr	Trp	Ala	_	Pro	Pro	Ser	Ile	10 Asp 25	Leu	Thr	Asn	Phe	15 Leu 30
25	Val	Arg	Tyr	Ser		Val	Lys	Asn	Glu		Asp	Val	Ala	Glu	Leu 45
	Ser	Ile	Ser	Pro	Ser 50	Asp	Asn	Ala	Val		Leu	Thr	Asn	Leu	
	Pro	Gly	Thr	Glu	Tyr 65	Val	Val	Ser	Val		Ser	Val	Tyr	Glu	Gln 75
30	His	Glu	Ser	Thr	Pro 80	Leu	Arg	Gly	Arg	Gln 85	Lys	Thr	Gly	Leu	
	Ser	Pro	Thr	Gly	Ile 95	Asp	Phe	Ser	Asp	Ile 100	Thr	Ala	Asn	Ser	Phe
					110	Ala				115			_	_	Arg
35					125	Glu				130					Asp 135
					140	Arg				145					150
40					155	Val				160					165
•					170	Leu				175					180
					185	Glu				190					195
45					200	Ala				205					210
					215	Thr				220					225
					230	Lys				235			-		240
50					245	Thr				250					255
					260	Ser				265					270
	Thr	GLu	Ile	Asp	Lys	Pro	Ser	Met	Ala	Asn	Glu	Gly	Leu	Asn	Gln

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275
                                                   280
              Pro Thr Asp Asp Ser Cys Phe Asp Pro Tyr Thr Val Ser His Tyr
                               290
                                                   295
              Ala Val Gly Asp Glu Trp Glu Arg Met Ser Glu Ser Gly Phe Lys
5
                               305
                                                   310
                                                                        315
              Leu Leu Cys Gln Cys Leu Gly Phe Gly Ser Gly His Phe Arg Cys
                               320
                                                   325
                                                                        330
              Asp Ser Ser Arg Trp Cys His Asp Asn Gly Val Asn Tyr Lys Ile
                              335
                                                   340
10
              Gly Glu Lys Trp Asp Arg Gln Gly Glu Asn Gly Gln Met Met Ser
                               350
                                                   355
              Cys Thr Cys Leu Gly Asn Gly Lys Gly Glu Phe Lys Cys Asp Pro
                               365
                                                   370
                                                                        375
              His Glu Ala Thr Cys Tyr Asp Asp Gly Lys Thr Tyr His Val Gly
                               380
                                                   385
15
              Glu Gln Trp Gln Lys Glu Tyr Leu Gly Ala Ile Cys Ser Cys Thr
                              395
                                                   400
              Cys Phe Gly Gly Gln Arg Gly Trp Arg Cys Asp Asn Cys Arg Arg
              Pro Gly
20
              (2) INFORMATION FOR SEQ ID NO: 13:
              (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 332
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
25
              (D) TOPOLOGY: linear
              (ii) MOLECULE TYPE: peptide
              (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:
              Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
30
              Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
                               20
                                                    25
              Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                               35
                                                    40
              Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
                               50
                                                    55
              Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                               65
                                                    70
              His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
                               80
                                                    85
              Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                               95
                                                   100
              Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
                              110
                                                   115
              Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
                              125
                                                   130
              Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
45
                              140
                                                   145
              Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
                              155
                                                   160
              Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
                              170
                                                   175
50
              Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
                              185
                                                   190
              Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
                              200
                                                   205
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Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
                              215
                                                  220
             Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
                              230
                                                  235
5
              Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
                              245
                                                  250
                                                                       255
             Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
                              260
                                                  265
                                                                       270
             Thr Glu Ile Asp Lys Pro Ser Met Ala Asn Ser Asp Ser Glu Cys
                              275
10
                                                  280
              Pro Leu Ser His Asp Gly Tyr Cys Leu His Asp Gly Val Cys Met
                              290
                                                  295
             Tyr Ile Glu Ala Leu Asp Lys Tyr Ala Cys Asn Cys Val Val Gly
                              305
                                                  310
                                                                       315
             Tyr Ile Gly Glu Arg Cys Gln Tyr Arg Asp Leu Lys Trp Trp Glu
15
                              320
                                                  325
             Leu Arg
              (2) INFORMATION FOR SEQ ID NO: 14:
              (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 341
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear
              (ii) MOLECULE TYPE: peptide
              (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:
25
              Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
                                                   10
              Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
                               20
                                                   25
             Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
30
                               35
                                                   40
              Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
                                                   55
              Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                               65
                                                   70
              His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
35
                              80
                                                   85
              Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                               95
                                                  100
             Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
                              110
                                                  115
40
              Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
                             125
                                                  130
                                                                       135
             Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
                              140
                                                  145
              Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
                              155
                                                  160
45
             Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
                              170
                                                  175
             Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
                             185
                                                  190
             Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
                              200
                                                  205
             Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
                              215
                                                  220
             Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
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					230					225					240
	Pro	Gly	Val	Asp		Thr	Ile	Thr	Val	235 Tyr 250	Ala	Val	Thr	Gly	240 Arg 255
5	Gly	Asp	Ser	Pro		Ser	Ser	Lys	Pro		Ser	Ile	Asn	Tyr	
	Thr	Glu	Ile	Asp	Lys 275	Pro	Ser	Met	Gly	Ile 280	Tyr	Ile	Ser	Gly	Met 285
	Ala	Pro	Arg	Pro	Ser 290	Leu	Thr	Lys	Lys	Gln 295	Arg	Phe	Arg	His	Arg 300
10	Asn	Arg	Lys	Gly	Tyr 305	Arg	Ser	Gln	Arg	Gly 310	His	Ser	Arg	Gly	Arg 315
	Asn	Gln	Asn	Ser	Arg 320	Arg	Pro	Ser	Arg	Ala 325	Met	Trp	Leu	Ser	Leu 330
15	Phe	Ser	Ser	Lys	Asn 335	Ser	Ser	Ser	Val	Pro 340	Ala				
20	(B) (C) (D) (ii)	SEQU LENC TYPE STRA TOPO	E: an ANDEI DLOGY LECUI	E CHA 446 mino ONESS C: 1:	acio s: si inear	TERIS ingle pept	STICS	5:	15: ID NO	D: 15	5:				
25	Pro 1	Thr	Asp	Leu	Arg 5	Phe	Thr	Asn	Ile	Gly 10	Pro	Asp	Thr	Met	Arg 15
	Val	Thr	Trp	Ala	Pro 20	Pro	Pro	Ser	Ile		Leu	Thr	Asn	Phe	
	Val	Arg	Tyr	Ser	Pro 35	Val	Lys	Asn	Glu	Glu 40	Asp	Val	Ala	Glu	Leu 45
30					50	_			Val	55					60
					65				Val	70					75
					80				Arg -	85			_		90
35					95				Asp	100					105
					110				Ala	115			_	_	120
40					125				Ser	130					135
					140				Ile Ile	145					150
					155				Gln	160				_	165
45					170			_	Ala	175					180
					185				Val	190					195
					200				Asn	205			-		210
50					215				Ala	220					225
					230				Val	235			_		240
	-			4											

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250
                               245
              Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
                               260
                                                   265
                                                                        270
              Thr Glu Ile Asp Lys Pro Ser Met Val Pro Gly Phe Lys Gly Asp
5
                               275
                                                   280
              Met Gly Leu Lys Gly Asp Arg Gly Glu Val Gly Gln Ile Gly Pro
                               290
                                                   295
              Arg Gly Xxx Asp Gly Pro Glu Gly Pro Lys Gly Arg Ala Gly Pro
                                                   310
                               305
                                                                        315
              Thr Gly Asp Pro Gly Pro Ser Gly Gln Ala Gly Glu Lys Gly Lys
10
                               320
                                                   325
              Leu Gly Val Pro Gly Leu Pro Gly Tyr Pro Gly Arg Gln Gly Pro
                               335
                                                   340
              Lys Gly Ser Thr Gly Phe Pro Gly Phe Pro Gly Ala Asn Gly Glu
                               350
                                                   355
15
              Lys Gly Ala Arg Gly Val Ala Gly Lys Pro Gly Pro Arg Gly Gln
                               365
                                                   370
              Arg Gly Pro Thr Gly Pro Arg Gly Ser Arg Gly Ala Arg Gly Pro
                               380
                                                   385
              Thr Gly Lys Pro Gly Pro Lys Gly Thr Ser Gly Gly Asp Gly Pro
                               395
                                                    400
              Pro Gly Pro Pro Gly Glu Arg Gly Pro Gln Gly Pro Gln Gly Pro
                               410
                                                    415
              Val Gly Phe Pro Gly Pro Lys Gly Pro Pro Gly Pro Pro Gly Arg
                               425
                                                   430
              Met Gly Cys Pro Gly His Pro Gly Gln Arg Gly
25
                               440
              (2) INFORMATION FOR SEQ ID NO: 16:
              (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 457
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
30
              (D) TOPOLOGY: linear
              (ii) MOLECULE TYPE: peptide
              (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:
              Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
35
              Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
                                                    25
              Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                                                    40
              Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
40
                                50
              Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln
                                65
                                                    70
              His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp
                                80
                                                    85
45
              Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe
                                95
                                                   100
              Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg
                              110
                                                   115
                                                                        120
              Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp
                              125
                                                   130
50
              Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr
                               140
                                                   145
              Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg
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155
                                                  160
              Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp
                              170
                                                  175
              Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu
5
                              185
                                                  190
              Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg
                              200
                                                  205
              Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe
                              215
                                                  220
                                                                       225
              Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys
10
                              230
                                                  235
              Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg
                              245
                                                  250
                                                       .
              Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg
                              260
                                                  265
15
              Thr Glu Ile Asp Lys Pro Ser Met Asn Val Ser Pro Pro Arg Arg
                              275
                                                  280
              Ala Arg Val Thr Asp Ala Thr Glu Thr Thr Ile Thr Ile Ser Trp
                              290
                                                  295
              Arg Thr Lys Thr Glu Thr Ile Thr Gly Phe Gln Val Asp Ala Val
20
                              305
                                                  310
              Pro Ala Asn Gly Gln Thr Pro Ile Gln Arg Thr Ile Lys Pro Asp
                              320
                                                  325
                                                                       330
              Val Arg Ser Tyr Thr Ile Thr Gly Leu Gln Pro Gly Thr Asp Tyr
                              335
                                                  340
                                                                       345
             Lys Ile Tyr Leu Tyr Thr Leu Asn Asp Asn Ala Arg Ser Ser Pro
25
                              350
                                                  355
              Val Val Ile Asp Ala Ser Thr Ala Ile Asp Ala Pro Ser Asn Leu
                              365
                                                  370
             Arg Phe Leu Ala Thr Thr Pro Asn Ser Leu Leu Val Ser Trp Gln
                              380
                                                  385
             Pro Pro Arg Ala Arg Ile Thr Gly Tyr Ile Ile Lys Tyr Glu Lys
30
                              395
                                                  400
              Pro Gly Ser Pro Pro Arg Glu Val Val Pro Arg Pro Arg Pro Gly
                              410
                                                  415
                                                                       420
             Val Thr Glu Ala Thr Ile Thr Gly Leu Glu Pro Gly Thr Glu Tyr
                              425
                                                 430
             Thr Ile Tyr Val Ile Ala Leu Lys Asn Asn Gln Lys Ser Glu Pro
                             440
                                                 445
                                                                       450
             Leu Ile Gly Arg Lys Lys Thr
                             455
              (2) INFORMATION FOR SEQ ID NO: 17:
40
              (i) SEQUENCE CHARACTERISTICS:
              (A) LENGTH: 368
              (B) TYPE: amino acid
              (C) STRANDEDNESS: single
              (D) TOPOLOGY: linear
              (ii) MOLECULE TYPE: peptide
45
              (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:
             Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
                                                   10
             Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu
50
                                                   25
             Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu
                              35
                                                   40
             Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu
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	Pro	s Gls	, Thi	r (1	50		,,,,	_		55	5				60
					0.3)				71)				60 Gln د 75
5					0 0)				25					د، Asp د 90
					, ,	,				1 6 3 1 1					Phe
					710					715					Arg
10					123					130					120 Asp
					140					Thr	Le				135 Thr
15	Pro	Gly	Thr	Glu	Tyr 155	Val	Val	Ser	Ile	Val	Ala	a Lei	ı Asr	ı Gly	150 Arg
75	Glu	Glu	Ser	Pro		Leu	Ile	Gly	Gln	160 Gln	Sei	r Thi	. Val	. Ser	165 Asp
	Val	Pro	Arg	Asp		Glu	Val	Val	Ala	175 Ala	Thi	r Pro	Thr	Ser	180 Leu
20				Trp	Asp										
20				Gly	Glu					205					010
				Gly	Ser					220					
25				Asp	Tyr					クマム					
				Pro											
				Asp											
30				Leu											
				Arg	200					745					~ ~ ~
				Ser											
35				Glu .											
				Tyr											
				Gly					273	355	A3 II	GIN	гàг	Ser	360
40					365	-3-	-,-	-11.							
	(2)	INFO	RMAT	ION :	FOR :	SEO :	א מז	0 - 1	ρ.						
	(1	SEQU LENG	FNCE	CHA	RACT	ERIS:	rics	:	•						
	(B)	TYPE	: am	ino a	acid										
45	(C)	STRAI	4DED	NESS:	: sin	ngle									
	(D)	TOPO	COGY	: lir	near										
	(xi)	SEQU	JENC:	E TYPE	PE: p SCRIE	pepti PTION	.de I: SI	EQ II	ОИО	: 18:	•				
50	Pro '	Thr A	lsp :	Leu A	lrg E	Phe T	hr A	Asn I	le (Gly H	Pro	Asp	Thr 1	Met 1	Arg
	Val '	Thr 1	rp /	Ala F	Pro E 20	Pro F	ro s	Ser I	le A	Asp I	Leu	Thr	Asn :	Phe I	15 Leu
	Val I	Arg 1	yr s	Ser P	Pro V	al L	ys A	sn G	lu e	25 Slu <i>F</i>	lsp	Val	Ala	3lu I	30 Seu

					35					40					45
					Ser 50					Val 55					Leu 60
5					65				Val	70					Gln 75
					80				Arg	85					90
					95				Asp	100					105
10					110				Ala	115	· ·		_	_	120
					125				Ser	130			_		135
15					140				Ile	145					150
15					155				·Ile	160				_	165
					170				Gln	175					180
20					185				Ala	190					195
					200				Val	205			_	_	210
					215				Asn	220					225
25					230				Ala	235					240
					245				Val	250				_	255
					260				Pro	265					270
30					275				Asn	280				_	285
					290				Thr	295					300
					305				Gly	310					315
35					320				Gln	325					330
					335				Leu	340					345
40					350			Asn	Asp	Asn 355	Ala	Arg	Ser	Ser	Pro 360
40	Val	Val	Ile	Asp	Ala 365	Ser	Thr								
	(2)	INFO	RMAT	OION	FOR	SEQ	ID N	10:	19:						
45			JENCE STH:		RACI	reris	STICS	5:							
4 5	(B)	TYPE	: an	nino	acio										
					inear	ingle	•								
	(ii)	MOI	ECUI	E TY	PE:	pept	ide	ero ·	ED NO	. 10	١.		-		
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Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg
1 5 10 15
Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu

Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Lu																
Ser 11e Ser		V = 1	Ara	Ture	50.2			T	7	C2			**- 1	~ ~	-1	30
Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Glu For Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Glu Glu For Glu His Glu Ser Thr Fro Leu Arg Gly Arg Gln Lys Thr Gly Leu Arg Glu Arg Fro Glu His Thr Ala Asn Ser Pro Thr Gly Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Arg 125 130 112 125 130 130 130 130 130 130 130 130 130 130						35					40					45
His Glu Ser Thr Fro Leu Arg Gly Arg Gln Lys Thr Gly Leu Arg Sly Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Pro Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg Ile Arg His His Fro Glu His Phe Ser Gly Arg Pro Arg Glu Arg Ile Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr Arg Val Pro Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg Pro Glu Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Arg Ile Val Ala Ala Thr Pro Thr Ser Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg Val Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lyg Gly Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lyg Gly Arg Ser Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg Arg Ser Pro Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg 245 So	5					50					55					60
His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Arg 800 800		Pro	Gly	Thr	Glu		Val	Val	Ser	Val		Ser	Val	Tyr	Glu	Gln 75
95		His	Glu	Ser	Thr		Leu	Arg	Gly	Arg		Lys	Thr	Gly	Leu	Asp 90
Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Are 110 110 115 115 116 116 116 Arg His His Pro Glu His Pro Ser Gly Arg Pro Arg Glu Arg Pro Arg Glu Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu The 140 155 160 160 160 160 160 160 160 160 160 160	10					95					100					105
11e Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Arg 125						110					115					Arg 120
Arg Val Fro His Ser Arg Asn Ser IIe Thr Leu Thr Asn Leu Thr 140 Pro Gly Thr Glu Tyr Val Val Ser IIe Val Ala Leu Asn Gly An 155 Glu Glu Ser Pro Leu Leu IIe Gly Gln Gln Ser Thr Val Ser As 170 Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu 185 Leu IIe Ser Trp Asp Ala Pro Ala Val Thr 200 Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Pro 215 Thr Val Pro Gly Ser Lys Ser Thr Ala Thr IIe Ser Gly Leu Ly 230 Pro Gly Val Asp Tyr Thr IIe Thr Val Tyr Ala Val Thr Gly Asn 245 Gly Asp Ser Pro Ala Ser Ser Lys Pro IIe Ser IIe Asn Tyr Ar 266 Thr Glu IIe Asp Lys Pro Ser Met Gly IIe Arg Gly Leu Lys Gly 275 Thr Lys Gly Glu Lys Gly Glu Asp Gly Phe Pro Gly Pro Pro Gly Gly Glu Asp Gly 285 Asp Met Gly IIe Lys Gly Asp Arg Gly Glu IIe Gly Pro Pro Gly Asp Gly 286 Pro Arg Gly Ser Ile Gly Pro Leu Gly Pro Pro Gly Arg Gly 387 40 Lys Leu Gly Val Pro Thr Gly Leu Pro Gly Lys Pro Gly Pro Arg Gl 388 45 Gln Arg Gly Pro Thr Gly Pro Lys Gly Asn Ser Gly Pro Arg Gl 396 Glu Lys Gly Gly Lys Pro Gly Pro Lys Gly Asn Gly Pro Arg Gl 397 198 45 Gln Arg Gly Pro Thr Gly Pro Lys Gly Asn Gly Pro Pro Gly Asp Gl 440 Pro Ala Gly Pro Pro Gly Fro Lys Gly Pro Pro Gly Pro Pro Gl 440 Pro Thr Gly Lys Pro Gly Pro Lys Gly Pro Pro Gly Pro Pro Gl 440		Ile	Arg	His	His		Glu	His	Phe	Ser		Arg	Pro	Arg	Glu	Asp 135
Pro Gly Thr Glu Tyr Val Val Ser II Val	15	Arg	Val	Pro	His		Arg	Asn	Ser	Ile		Leu	Thr	Asn	Leu	Thr 150
Silva Ser Pro Leu Leu Ile Silva Ser Thr Val Ser And Nal Pro Arg Asp Leu Silva Arg Arg Tyr Arg Arg Arg Leu Silva Arg Arg Tyr Arg Ar		Pro	Gly	Thr	Glu	Tyr 155	Val	Val	Ser	Ile	Val	Ala	Leu	Asn	Gly	Arg 165
Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu Ile Ser Trp Asp Asp Ala Pro Ala Val Ala Ala Trp Val Arg Tyr Tyr Asp 200 205	20	Glu	Glu	Ser	Pro		Leu	Ile	Gly	Gln		Ser	Thr	Val	Ser	Asp 180
Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg 200 200 205		Val	Pro	Arg	Asp	Leu 185	Glu	Val	Val	Ala	Ala	Thr	Pro	Thr	Ser	Leu 195
File Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Pro 220						200					205			-	_	Arg
Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Ly 230 235 25 26 27 28 28 28 28 29 29 29 29 29 29 29 29 29 29 29 29 29	25					215					220					Phe 225
9ro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Ar 245		Thr	Val	Pro	Gly	Ser 230	Lys	Ser	Thr	Ala		Ile	Ser	Gly	Leu	Lys 240
30 Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Ar 260		Pro	Gly	Val	Asp	Tyr 245	Thr	Ile	Thr	Val		Ala	Val	Thr	Gly	Arg 255
Thr Glu Ile Asp Lys Pro Ser Met Gly Ile Arg Gly Leu Lys Gly Thr Lys Gly Glu Lys Gly Glu Asp Gly Pro Gly Pro Gly Pro Gly Glu Asp Gly Glu Ile Gly Pro Pro Gly Gly Glu Asp Gly Glu Ile Gly Pro Pro Gly Gly Glu Glu Gly Glu Glu Gly Glu Gly Glu Gly Glu Gly	30					260					265				_	Arg 270
## Lys Gly Glu Lys Gly Glu Asp Gly Phe Pro Gly Phe Lys Gly 290 ## Asp Met Gly Ile Lys Gly Asp Arg Gly Glu Ile Gly Pro Pro Gly 300 ## Arg Gly Glu Asp Gly Pro Glu Gly Pro Lys Gly Arg Gly Glu 310 ## Arg Gly Glu Asp Gly Pro Glu Gly Pro Lys Gly Arg Gly Glu Lys Gly Arg Gly Glu 325 ## Ash Gly Asp Pro Gly Pro Leu Gly Pro Pro Gly Glu Lys Gly Arg Gln Gly 335 ## Ash Gly Asp Pro Gly Leu Pro Gly Tyr Pro Gly Arg Gln Gly 350 ## Ash Gly Ser Ile Gly Phe Pro Gly Phe Pro Gly Ala Ash Gly 360 ## Arg Gly Gly Arg Gly Thr Pro Gly Lys Pro Gly Pro Arg Gly Glu Arg Gly Pro Arg Gly Fro Arg Gly Glu Arg Gly Pro Arg Gly Pro Arg Gly Glu Arg Gly Pro Arg Gly Pro Arg Gly Fro Arg Gly Pro Pro Gly Pro						275					280					Gly 285
Asp Met Gly Ile Lys Gly Asp Arg Gly Glu Ile Gly Pro Pro Gl 305		Thr	Lys	Gly	Glu	Lys 290	Gly	Glu	Asp	Gly		Pro	Gly	Phe	Lys	Gly 300
Pro Arg Gly Glu Asp Gly Pro Glu Gly Pro Lys Gly Arg Gly Glu 325 325 335 3340 340 340 340 340 340 340 340 340 34	35	Asp	Met	Gly	Ile	Lys 305	Gly	Asp	Arg	Gly	Glu 310	Ile	Gly	Pro	Pro	Gly 315
40		Pro	Arg	Gly	Glu	Asp 320	Gly	Pro	Glu	Gly	Pro	Lys	Gly	Arg	Gly	Gly 330
Lys Leu Gly Val Pro Gly Leu Pro Gly Tyr Pro Gly Arg Gln Gl 350 Pro Lys Gly Ser Ile Gly Phe Pro Gly Phe Pro Gly Ala Asn Gl 365 Glu Lys Gly Gly Arg Gly Thr Pro Gly Lys Pro Gly Pro Arg Gl 380 Gln Arg Gly Pro Thr Gly Pro Arg Gly Glu Arg Gly Pro Arg Gl 395 Gln Arg Gly Pro Thr Gly Pro Lys Gly Asn Ser Gly Gly Asp Gl 400 Ile Thr Gly Lys Pro Gly Gly Pro Lys Gly Pro Asn Gly Pro Gln Gl 410 Pro Ala Gly Pro Pro Gly Gly Arg Gly Pro Asn Gly Pro Gln Gl 425 Pro Thr Gly Phe Pro Gly Pro Lys Gly Pro Pro Gly Pro Pro Gl 440 Pro Thr Gly Phe Pro Gly Pro Lys Gly Pro Pro Gly Pro Pro Gl		Pro	Asn	Gly	Asp	Pro 335	Gly	Pro	Leu	Gly		Pro	Gly	Glu	Lys	Gly 345
Pro Lys Gly Ser Ile Gly Phe Pro Gly Phe Pro Gly Ala Asn Gl 365 370 370 Glu Lys Gly Gly Arg Gly Thr Pro Gly Lys Pro Gly Pro Arg Gl 380 385 385 Gln Arg Gly Pro Thr Gly Pro Arg Gly Glu Arg Gly Pro Arg Gl 395 400 Ile Thr Gly Lys Pro Gly Pro Lys Gly Asn Ser Gly Gly Asp Gl 410 415 Pro Ala Gly Pro Pro Gly Glu Arg Gly Pro Asn Gly Pro Gln Gl 425 Pro Thr Gly Phe Pro Gly Pro Lys Gly Pro Pro Gly Pro Pro Gl 440 Pro Thr Gly Phe Pro Gly Pro Lys Gly Pro Pro Gly Pro Pro Gl	40					350					355					Gly 360
Glu Lys Gly Gly Arg Gly Thr Pro Gly Lys Pro Gly Pro Arg Gl 380						365					Phe 370					Gly
Gln Arg Gly Pro Thr Gly Pro Arg Gly Glu Arg Gly Pro Arg Gl 395 400 40 Ile Thr Gly Lys Pro Gly Pro Lys Gly Asn Ser Gly Gly Asp Gl 410 415 415 Pro Ala Gly Pro Pro Gly Glu Arg Gly Pro Asn Gly Pro Gln Gl 425 430 Pro Thr Gly Phe Pro Gly Pro Lys Gly Pro Pro Gly Pro Pro Gl 440 445	45					380					Lys 385					Gly
Ile Thr Gly Lys Pro Gly Pro Lys Gly Asn Ser Gly Gly Asp Gl 410 415 42 Pro Ala Gly Pro Pro Gly Glu Arg Gly Pro Asn Gly Pro Gln Gl 425 430 Pro Thr Gly Phe Pro Gly Pro Lys Gly Pro Pro Gly Pro Pro Gl 440 445	70	Gln	Arg	Gly	Pro	Thr 395	Gly	Pro	Arg	Gly	Glu	Arg	Gly	Pro	Arg	Gly
Pro Ala Gly Pro Pro Gly Glu Arg Gly Pro Asn Gly Pro Gln Gl 425 430 430 Pro Thr Gly Phe Pro Gly Pro Lys Gly Pro Pro Gly Pro Pro Gl 440 445		Ile	Thr	Gly	Lys	Pro	Gly	Pro	Lys	Gly	Asn	Ser	Gly	Gly	Asp	Gly
Pro Thr Gly Phe Pro Gly Pro Lys Gly Pro Pro Gly Pro Pro Gly 440	50	Pro	Ala	Gly	Pro	Pro	Gly	Glu	Arg	Gly	Pro	Asn	Gly	Pro	Gln	
		Pro	Thr	Gly	Phe	Pro	Gly	Pro	Lys	Gly	Pro	Pro	Gly	Pro	Pro	
		Lys	Asp	Gly	Leu		Gly	His	Pro	Gly	Gln	Arg	Gly	Glu	Thr	450

(2) INFORMATION FOR SEQ ID NO: 20: (i) SEQUENCE CHARACTERISTICS: .5 (A) LENGTH: 432 (B) TYPE: amino acid (C) STRANDEDNESS: single (D) TOPOLOGY: linear (ii) MOLECULE TYPE: peptide (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20: Pro Thr Asp Leu Arg Phe Thr Asn Ile Gly Pro Asp Thr Met Arg Val Thr Trp Ala Pro Pro Pro Ser Ile Asp Leu Thr Asn Phe Leu Val Arg Tyr Ser Pro Val Lys Asn Glu Glu Asp Val Ala Glu Leu Ser Ile Ser Pro Ser Asp Asn Ala Val Val Leu Thr Asn Leu Leu Pro Gly Thr Glu Tyr Val Val Ser Val Ser Ser Val Tyr Glu Gln His Glu Ser Thr Pro Leu Arg Gly Arg Gln Lys Thr Gly Leu Asp Ser Pro Thr Gly Ile Asp Phe Ser Asp Ile Thr Ala Asn Ser Phe Thr Val His Trp Ile Ala Pro Arg Ala Thr Ile Thr Gly Tyr Arg Ile Arg His His Pro Glu His Phe Ser Gly Arg Pro Arg Glu Asp Arg Val Pro His Ser Arg Asn Ser Ile Thr Leu Thr Asn Leu Thr Pro Gly Thr Glu Tyr Val Val Ser Ile Val Ala Leu Asn Gly Arg Glu Glu Ser Pro Leu Leu Ile Gly Gln Gln Ser Thr Val Ser Asp Val Pro Arg Asp Leu Glu Val Val Ala Ala Thr Pro Thr Ser Leu Leu Ile Ser Trp Asp Ala Pro Ala Val Thr Val Arg Tyr Tyr Arg Ile Thr Tyr Gly Glu Thr Gly Gly Asn Ser Pro Val Gln Glu Phe Thr Val Pro Gly Ser Lys Ser Thr Ala Thr Ile Ser Gly Leu Lys Pro Gly Val Asp Tyr Thr Ile Thr Val Tyr Ala Val Thr Gly Arg Gly Asp Ser Pro Ala Ser Ser Lys Pro Ile Ser Ile Asn Tyr Arg Thr Glu Ile Asp Lys Pro Ser Met Ala Ala Gly Ser Ile Thr Thr

290 295 300

Gly His Phe Lys Asp Pro Lys Arg Leu Tyr Cys Lys Asn Gly Gly
305 310 315

Phe Phe Leu Arg Ile His Pro Asp Gly Arg Val Asp Gly Val Arg
320 325 330

Glu Lys Ser Asp Pro His Ile Lys Leu Gln Leu Gln Ala Glu Glu
335 340 345

Arg Gly Val Val Ser Ile Lys Gly Val Cys Ala Asn Arg Tyr Leu

Leu Pro Ala Leu Pro Glu Asp Gly Gly Ser Gly Ala Phe Pro Pro

	21-	14-4	.	61	350	~1	_	_		355	_	_			360
					365					370	Ser				375
5					380					385				_	Asn 390
	Thr	Tyr	Arg	Ser	Arg 395	Lys	Tyr	Thr	Ser	Trp	Tyr	Val	Ala	Leu	Lys 405
	Arg	Thr	Gly	Gln	Tyr 410	Lys	Leu	Gly	Ser	Lys 415	Thr	Gly	Pro	Gly	Gln 420
10	Lys	Ala	Ile	Leu	Phe 425	Leu	Pro	Met	Ser		Lys	Ser			120
15	(i) (A) (B) (C) (D) (ii)	SEQUENCE TYPE STRATE TOPO	JENCH GTH: E: ar ANDEH DLOGY LECUI	E CHI 574 mino DNES: Y: 1:	acio s: s: inea: YPE:	reni: d ingle r pept	STIC e tide		21: ID NO	D: 2]	1:				
20	Pro	Thr	Asp	Leu	Arg	Phe	Thr	Asn	Ile	Gly	Pro	Asp	Thr	Met	Arg
	1				5					10	Leu				15
					20					25	Asp				30
25					35					40	Leu				45
					50					55	Ser				60
					65					70	Lys				75
30					80					85	Thr				90
					95					100	Ile				105
					110					115					120
35					125					130	Arg -				135
					140					145	Leu				150
40					155					160	Ala			_	165
40					170					175	Ser				180
					185					190	Thr				195
45	Leu	Ile	Ser	Trp	Asp 200	Ala	Pro	Ala	Val	Thr 205	Val	Arg	Tyr	Tyr	Arg 210
43	Ile	Thr	Tyr	Gly	Glu 215	Thr	Gly	Gly	Asn	Ser 220	Pro	Val	Gln	Glu	Phe
	Thr	Val	Pro	Gly		Lys	Ser	Thr	Ala	Thr 235	Ile	Ser	Gly	Leu	
50	Pro	Gly	Val	Asp		Thr	Ile	Thr	Val	Tyr 250	Ala	Val	Thr	Gly	
	Gly	Asp	Ser	Pro		Ser	Ser	Lys	Pro	Ile	Ser	Ile	Asn	Tyr	
	Thr	Glu	Ile	Asp		Pro	Ser	Met	Ala	265 Ile	Pro	Ala	Pro	Thr	270 Asp

	Leu	Lys	Phe	Thr	275 Gln	Val	Thr	Pro	Thr	280 Ser	Leu	Ser	Ala	Gln	285 Trp
					290					295					300
5					305					310	Arg		_		315
					320					325	Ile				330
10					335					340	Met				345
					350					355	Thr				360
	Pro	Ala	Gln	Gly	Val 365	Val	Thr	Thr	Leu	Glu 370	Asn	Val	Ser	Pro	Pro 375
15					380					385	Thr				390
					395					400	Gly				405
20					410					415	Gln				420
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25					455					460	Ile				465
					470					475	Ser				480
30					485					490	Tyr			_	495
					500					505	Val				510
					515					520	Leu			_	525
35					530					535	Asn			_	540
					545					550	Glu				555
40					His 560	Pro	Asn	Leu	His	Gly 565	Pro	Glu	Ile	Leu	Asp 570
	Val	Pro	Ser	Thr											

Claims

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- In a method for production of transfected cells by transferring a foreign gene into target cells using a perforation method, said method for production of cells transfected with a foreign gene which comprises a step of, after injection of a foreign gene into target cells using a perforation method, culturing the cells in the presence of a cell-adhering active substance.
- 2. The method for production of transfected cells according to claim 1, the culturing step is a step of culturing using a culture wear covered with a cell-adhering active substance.
- 3. The method for production of transfected cells according to claim 1, wherein the cell-adhering active substance is a cell-adhering active polypeptide or a functional equivalent of said polypeptide.
- 4. The method for production of transfected cells according to claim 3, wherein the cell-adhering active polypeptide is

a cell-adhering and/or cell-spreading active polypeptide.

- 5. The method for production of transfected cells according to claim 3, wherein the cell-adhering and/or cell-spreading active polypeptide is a polypeptide containing the amino acid sequence represented by SEQ ID: No. 1 and/or the amino acid sequence represented by SEQ ID: No. 2.
- The method for production of transfected cells according to claim 3, wherein the cell-adhering active polypeptide is selected from polypeptides represented by SEQ ID: Nos. 3, 4 and 5.
- 7. The method for production of transfected cells according to claim 1, wherein the cell-adhering active substance is poly-N-p-vinylbenzyl-D-lactoneamide.
 - 8. The method for production of transfected cells according to claim 1, wherein the target cells are selected from hematopoiesis stem cell, peripheral blood stem cell, umbilical blood cell, ES cell, lymphocyte and cancer cell.
 - 9. The method for production of transfected cells according to claim 1, wherein the foreign gene is nucleic acid selected from nucleic acids encoding proteins, nucleic acids encoding polypeptides, antisense DNA's, antisense RNA's, ribozymes, nucleic acids encoding intracellular antibodies and pseudogenes (decoy genes).
- 10. The method for production of transfected cells according to claim 1, wherein the foreign gene is nucleic acid selected from nucleic acids encoding proteins, nucleic acids encoding polypeptides, antisense DNA's, antisense RNA's, ribozymes, nucleic acids encoding intracellular antibodies and pseudogenes (decoy genes) and the nucleic acid is incorporated into the vector.
- 25 11. The method for production of transfected cells according to claim 1, wherein the vector is a vector selected from retrovirus vector, adenovirus vector, vacciniavirus vector and herpesvirus vector.
 - 12. The method for production of transfected cells according to claim 1, the perforation method is selected from an electroporation method, a microinjection method and a particle gun method.
 - 13. Transfected cells produced by a method for production of transfected cells according to claim 1.
 - 14. A kit for production of transfected cells with a foreign gene which is used in a method for production of transfected cells according to claim 1, said kit comprises containing a cell-adhering active substance.

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Fig. 1

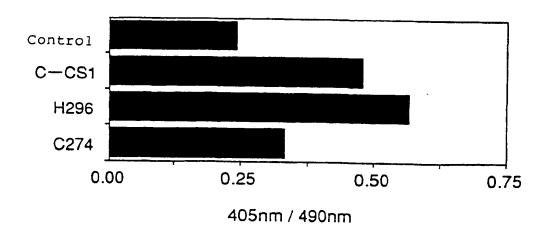
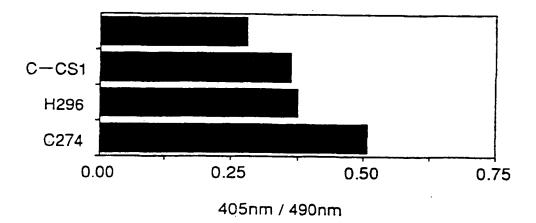


Fig. 2



INTERNATIONAL SEARCH REPORT International application No. PCT/JP95/02425 A. CLASSIFICATION OF SUBJECT MATTER Int. C16 C12N15/87, C12N5/10, C07K14/78 According to International Patent Classification (IPC) or to both national classification and IPC FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) Int. $C1^6$ C12N15/87, C12N5/10, C07K14/78 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base comulted during the international search (name of data base and, where practicable, search terms used) WPI, WPI/L, BIOSIS PREVIEWS CAS ONLINE C. DOCUMENTS CONSIDERED TO BE RELEVANT Citation of document, with indication, where appropriate, of the relevant passages Category* Relevant to claim No. JP, 4-063597, A (W.R. Grace & Co.), 1 - 14Α February 28, 1992 (28. 02. 92) & EP, 463508, A & CA, 2044307, A JP, 6-090771, A (Shiseido Co., Ltd.), 1 - 14Α April 5, 1994 (05. 04. 94) (Family: none) Further documents are listed in the continuation of Box C. See patent family annex. later document published after the international filing date or priority date and not in conflict with the application but cited to understand Special categories of cited documents: "A" document defining the general state of the art which is not considered to be of particular relevance the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be "E" cartier document but published on or after the international filing date considered novel or cannot be considered to involve an inventive step when the document is taken alone "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is "O" document referring to an oral disclosure, use, exhibition or other combined with one or more other such documents, such combination being obvious to a person skilled in the art "P" document published prior to the international filing date but later than the priority date claimed "&" document member of the same patent family Date of the actual completion of the international search Date of mailing of the international search report March 1, 1996 (01. 03. 96) March 19, 1996 (19, 03, 96) Authorized officer Name and mailing address of the ISA/ Japanese Patent Office Telephone No. Facsimile No.

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